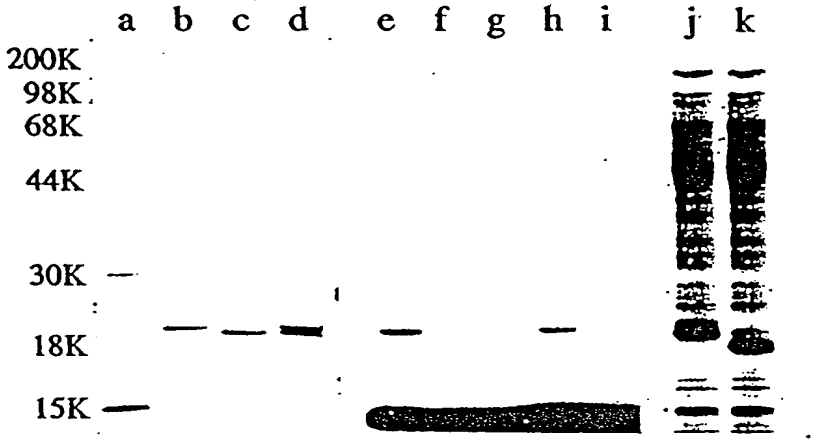
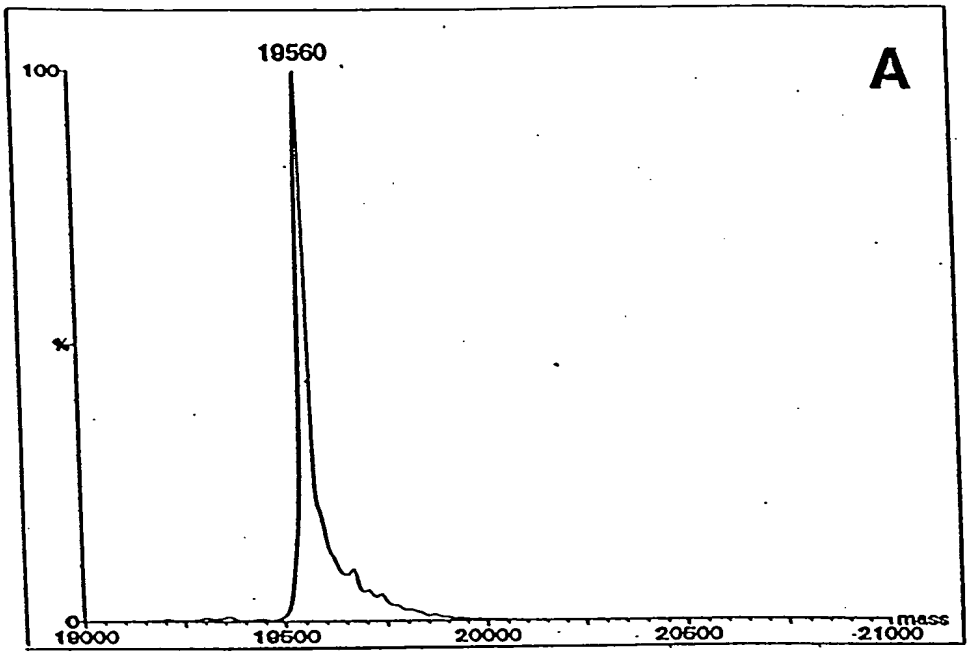


FIG. 1


19560
100
%
0
A
19000
19500
20000
20500
21000
mass

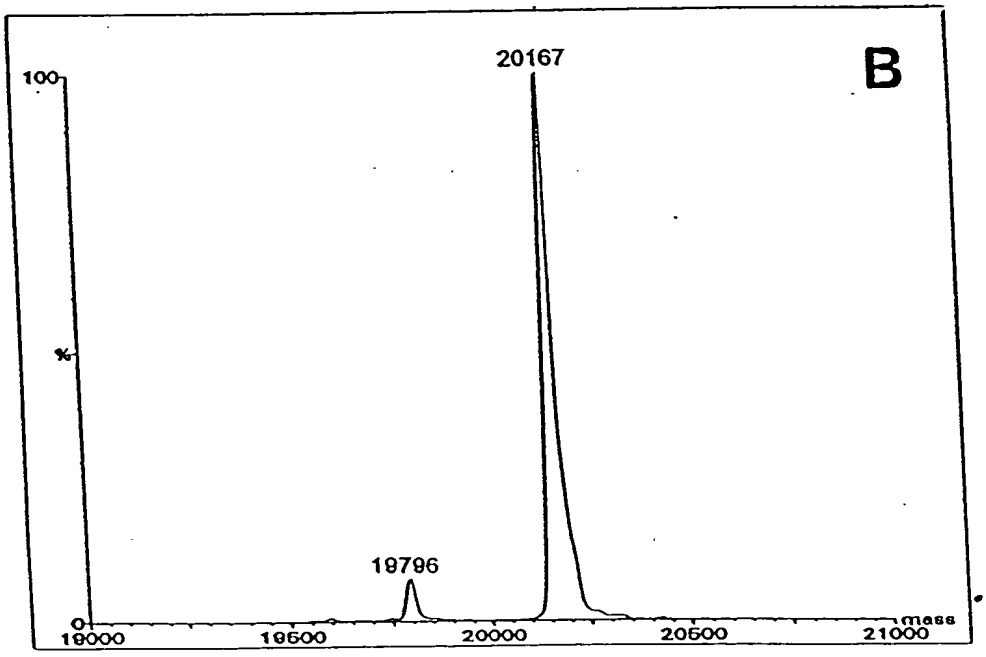

20167
100
%
0
B
19796
19000
19500
20000
20500
21000
mass

FIG. 2

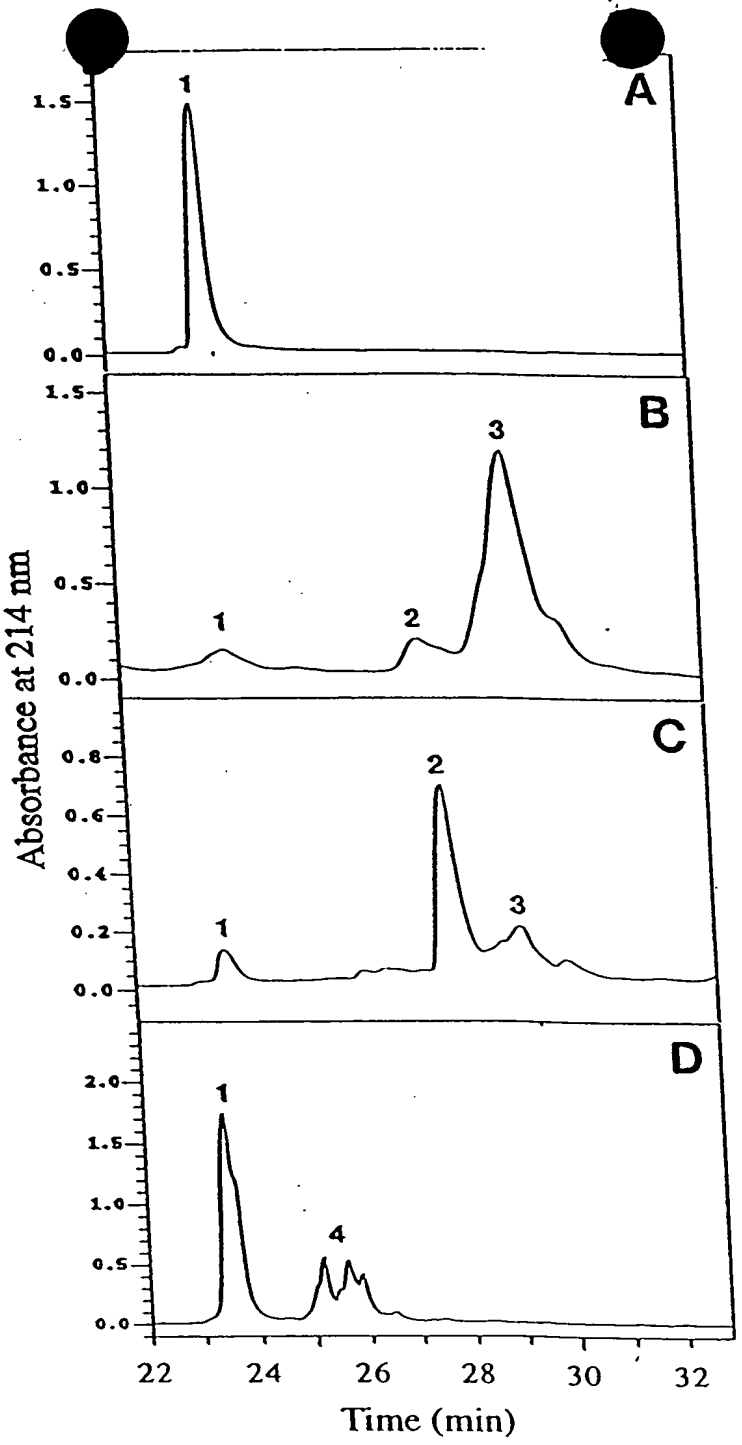

A
B
C
D
1
2
3
4
Absorbance at 214 nm
Time (min)
0.0
0.5
1.0
1.5
2.0
0.2
0.4
0.6
0.8
22
24
26
28
30
32

FIG. 3

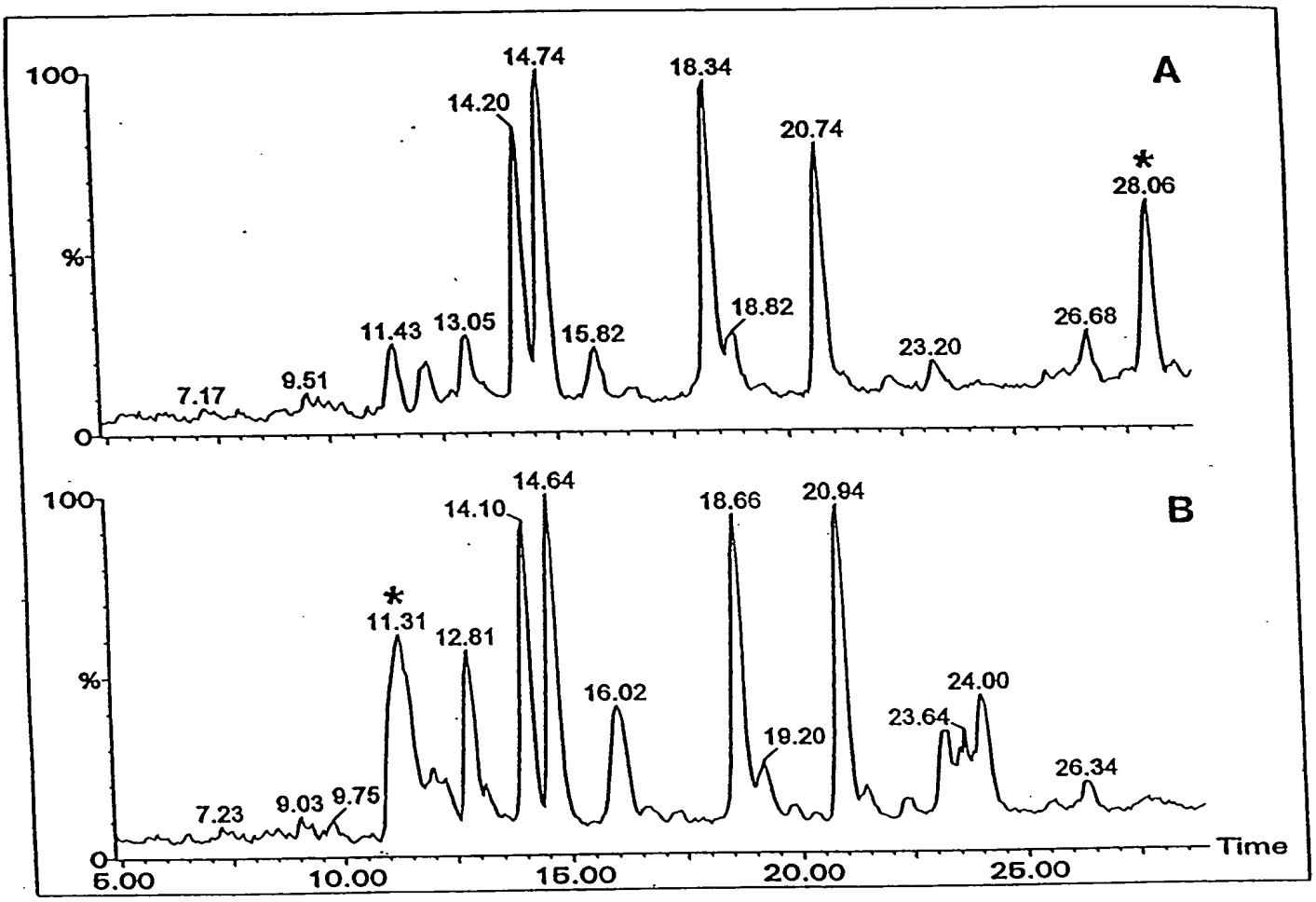

A
100
%
0
14.74
14.20
18.34
20.74
*
28.06
18.82
11.43
13.05
15.82
26.68
23.20
7.17
9.51
B
100
%
0
14.64
14.10
18.66
20.94
*
11.31
12.81
16.02
24.00
23.64
19.20
26.34
7.23
9.03
9.75
Time
5.00
10.00
15.00
20.00
25.00

FIG. 4

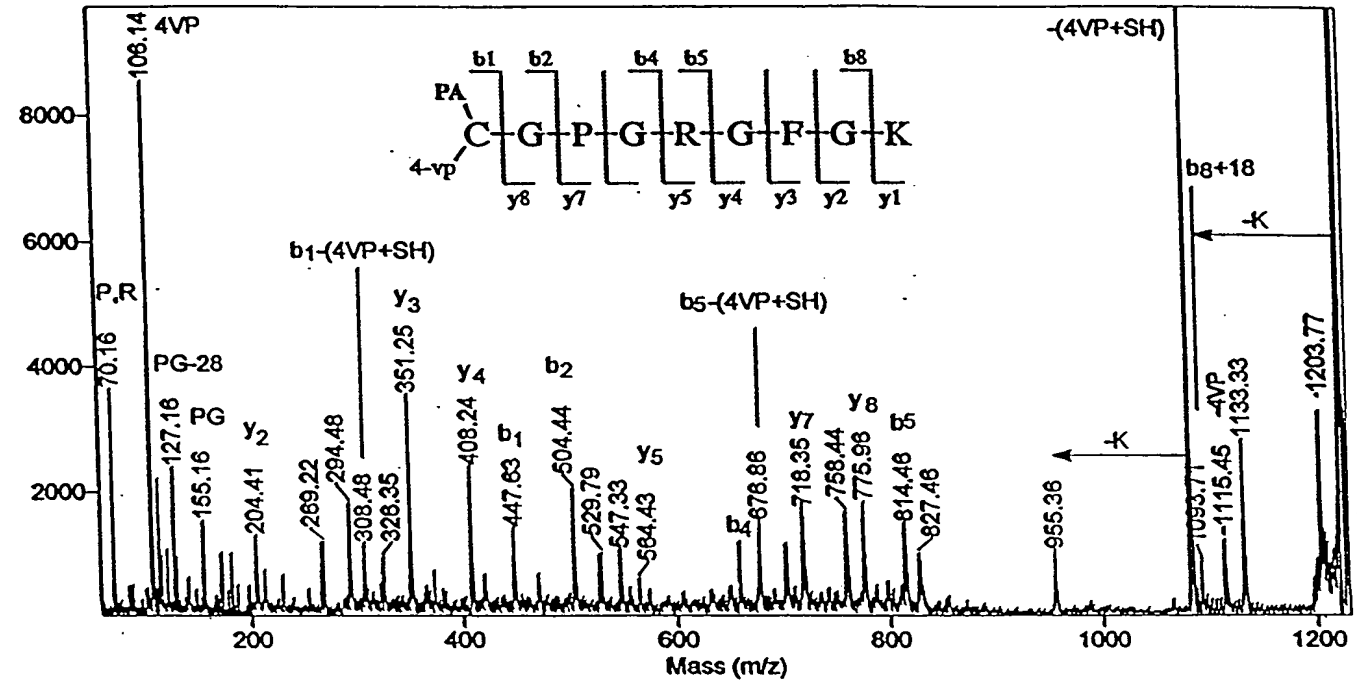
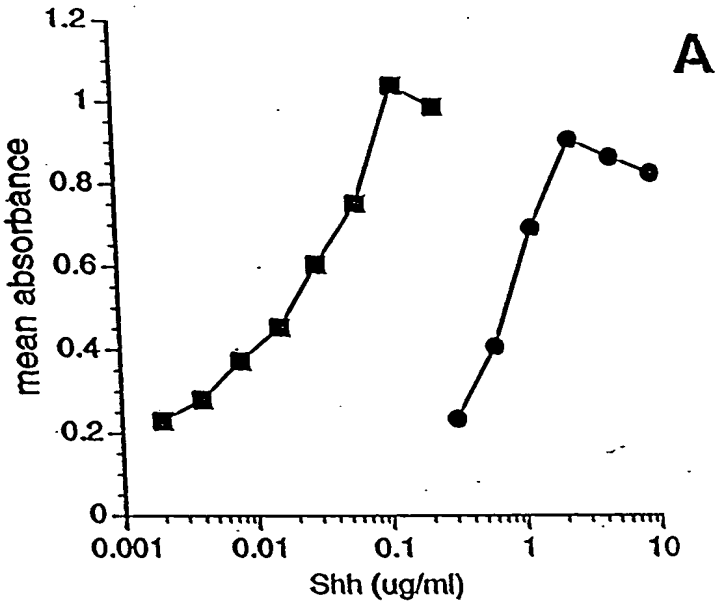

4VP
106.14
-(4VP+SH)
b1 b2 b4 b5 b8
PA
C G P G R G F G K
4-vp
y8 y7 y5 y4 y3 y2 y1
b8+18
-K
b1-(4VP+SH)
b5-(4VP+SH)
P,R
70.16
PG-28
PG
127.18
155.16
204.41
269.22
294.48
308.48
328.35
351.25
408.24
447.63
504.44
529.79
547.33
584.43
678.86
718.35
758.44
775.98
814.48
827.46
955.36
1093.71
1115.45
4VP
1133.33
1203.77
Counts
8000
6000
4000
2000
200
400
600
800
1000
1200
Mass (m/z)

FIG. 5


A
mean absorbance
1.2
1
0.8
0.6
0.4
0.2
0
0.001
0.01
0.1
1
10
Shh (ug/ml)

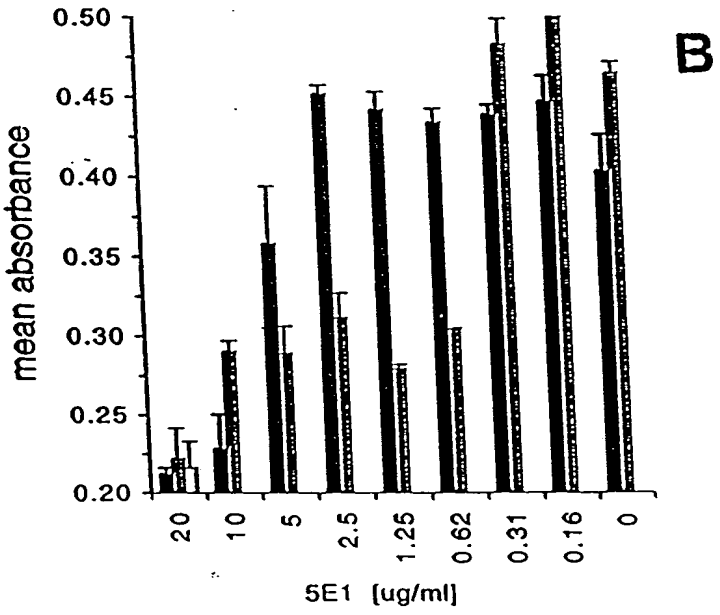

B
mean absorbance
0.50
0.45
0.40
0.35
0.30
0.25
0.20
20
10
5
2.5
1.25
0.62
0.31
0.16
0
5E1 [ug/ml]

FIG. 6

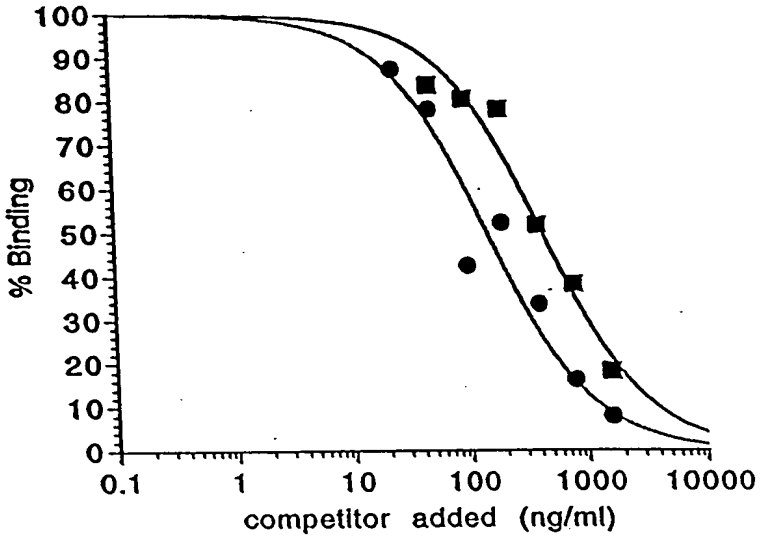


FIG-7

0009259 0696259

Figure 8: Alignment of N-terminal fragments of Human Hedgehog Proteins

```
          1                                                        50
Indian    CGPGRVVGSR RRPPRK-LVP LAYKQFSPNV PEKTLGASGR YEGKIARSSE
Sonic     CGPGRGFG-K RRHPKK-LTP LAYKQFIPNV AEKTLGASGR YEGKISRNSE
Desert    CGPGRGPVGR RRYARKQLVP LLYKQFVPGV PERTLGASGP AEGRVARGSE

          51                                                      100
Indian    RFKELTPNYN PDIIFKDEEN TGADRLMTQR CKDRLNSLAI SVMNQWPGVK
Sonic     RFKELTPNYN PDIIFKDEEN TGADRLMTQR CKDKLNALAI SVMNQWPGVK
Desert    RFRDLVPNYN PDIIFKDEEN SGADRLMTER CKERVNALAI AVMNMWPGVR

          101                                                     150
Indian    LRVTEGWDED GHHSEESLHY EGRAVDITTS DRDRNKYGLL ARLAVEAGFD
Sonic     LRVTEGWDED GHHSEESLHY EGRAVDITTS DRDRSKYGML ARLAVEAGFD
Desert    LRVTEGWDED GHHAQDSLHY EGRALDITTS DRDRNKYGLL ARLAVEAGFD

          151                       176
Indian    WVYYESKAHV HCSVKSEHSA AAKTGG     SEQ ID NO: 1
Sonic     WVYYESKAHI HCSVKAENSV AAKSGG     SEQ ID NO. 2
Desert    WVYYESRNHV HVSVKADNSL AVRAGG     SEQ ID NO. 3
```

Gap(s), indicated by -, added to facilitate alignment

0605360 : 0605360

Figure 9: Consensus Sequence of N-terminal Fragments

SEQ ID NO. 4

1 40

$CGPGR_{X1\ X2\ X3\ X4\ X5}$ [redacted] $R_{X6\ X7\ X8}K_{X9}L_{X10}P$ $L_{X11}YKQF_{X12}P_{X13}V$ [redacted] $_{X14}EKTLGASGR$

80

$_{X15}EGK_{X16\ X17}R_{X18}SE$ $RFK_{X19}L_{X20}PNYN$ $PDIIFKDEEN$ $_{X21}GADRLMT_{X22}R$

120

$CK_{X23\ X24X25}NSLAI$ $_{X26}VMN_{X27}WPGVK$ $LRVTEGWDED$ $GHH_{X28\ X29\ X30}SLHY$

160

$EGRAVDITTS$ $DRDR_{X31}KYG_{X32}L$ $ARLAVEAGFD$ $WVYYES_{X33\ X34}H_{X35}$

176

$H_{X36}SVK_{X37\ X38\ X39}S_{X40}$ $AA_{X41\ X42}GG$

Where:
X1 is either V or G;
X2 is either V, F or P;
X3 is either G or V;
X4 is either S or G;
X5 is either R or K;
X6 is either P, H or Y;
X7 is either P or A;
X8 is either R or K;
X9 is any amino acid;
X10 is either V or T;
X11 is either A or L;
X12 is either S, I or V;
X13 is either N or G;
X14 is either P or A;
X15 is either Y or A;
X16 is either I or V;
X17 is either A or S;
X18 is either S, N or G;
X19 is either E or D;
X20 is either T or V;
X21 is either T or S;
X22 is either Q or E;
X23 is either D or E;
X24 is either R or K;
X25 is either L or V;
X26 is either S or A;
X27 is either Q or M;
X28 is either S or A;
X29 is either E or Q;
X30 is either E or D;
X31 is either N or S;
X32 is either L or M;
X33 is either K or R;
X34 is either A or N;
X35 is either V or I;
X36 is either C or V;
X37 is either S or A;
X38 is either E or D;
X39 is either H or N;
X40 is either A, V or L;
X41 is either K or R; and
X42 is either T, S or A.

Induction of alkaline phosphatase in 10T1/2 cells by acyl-hedgehog. Effect of acyl chain length.

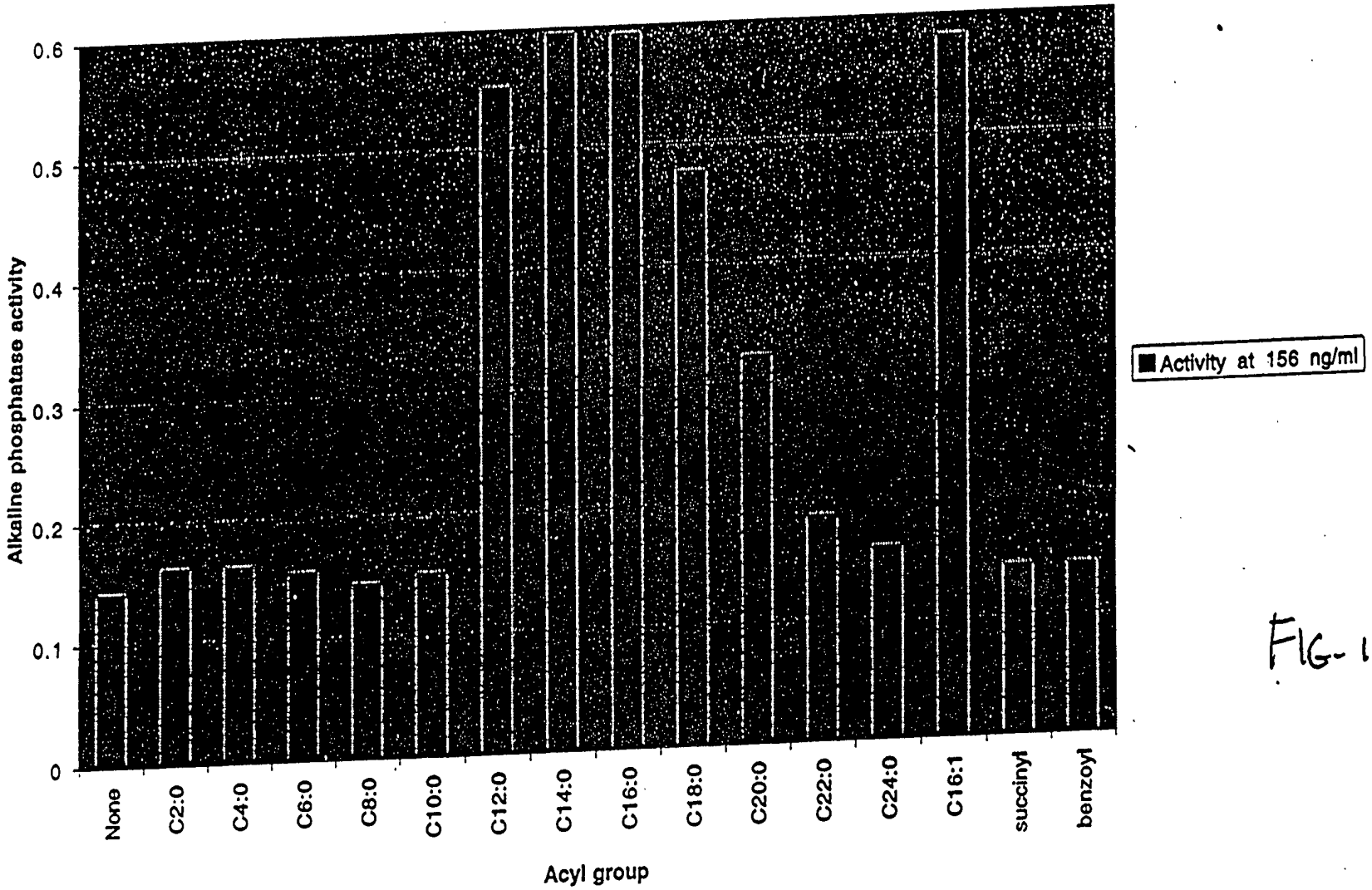
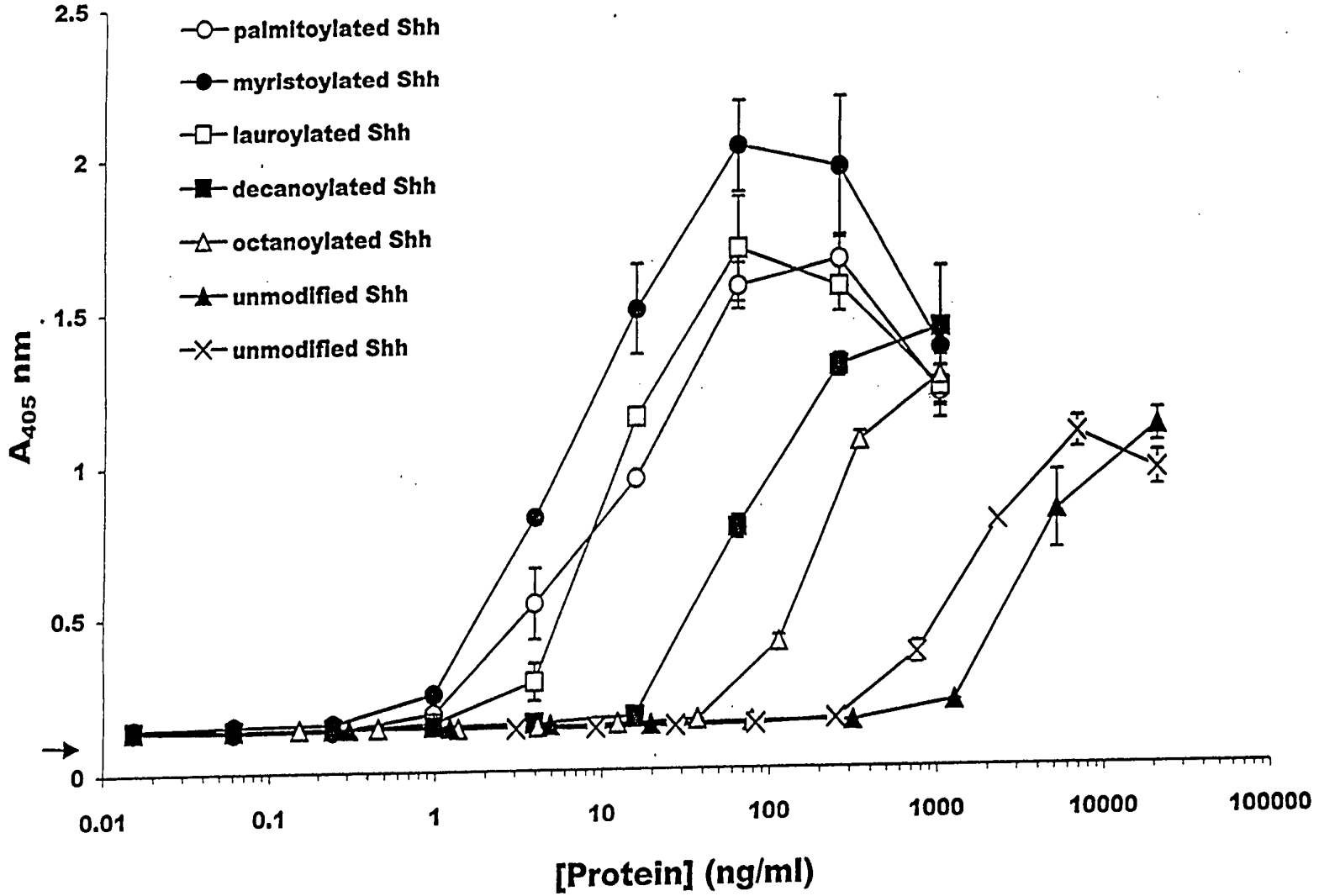


FIG-1D

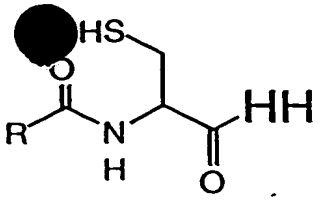
09579660 .052600

Figure 11

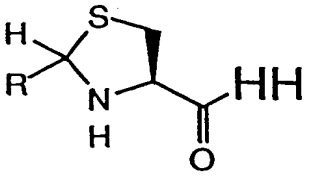


0957980 .052606

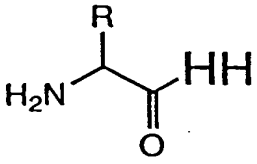
A



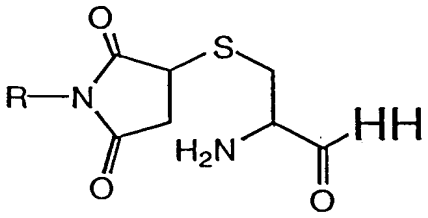
B



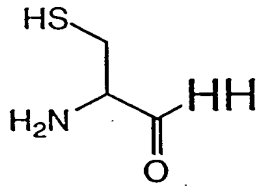
C



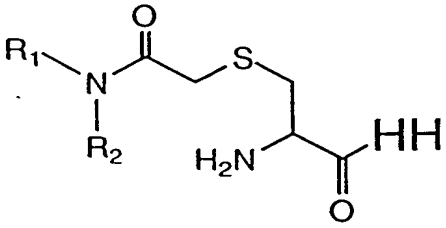
D



E



F



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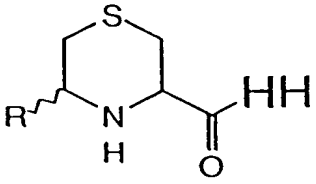
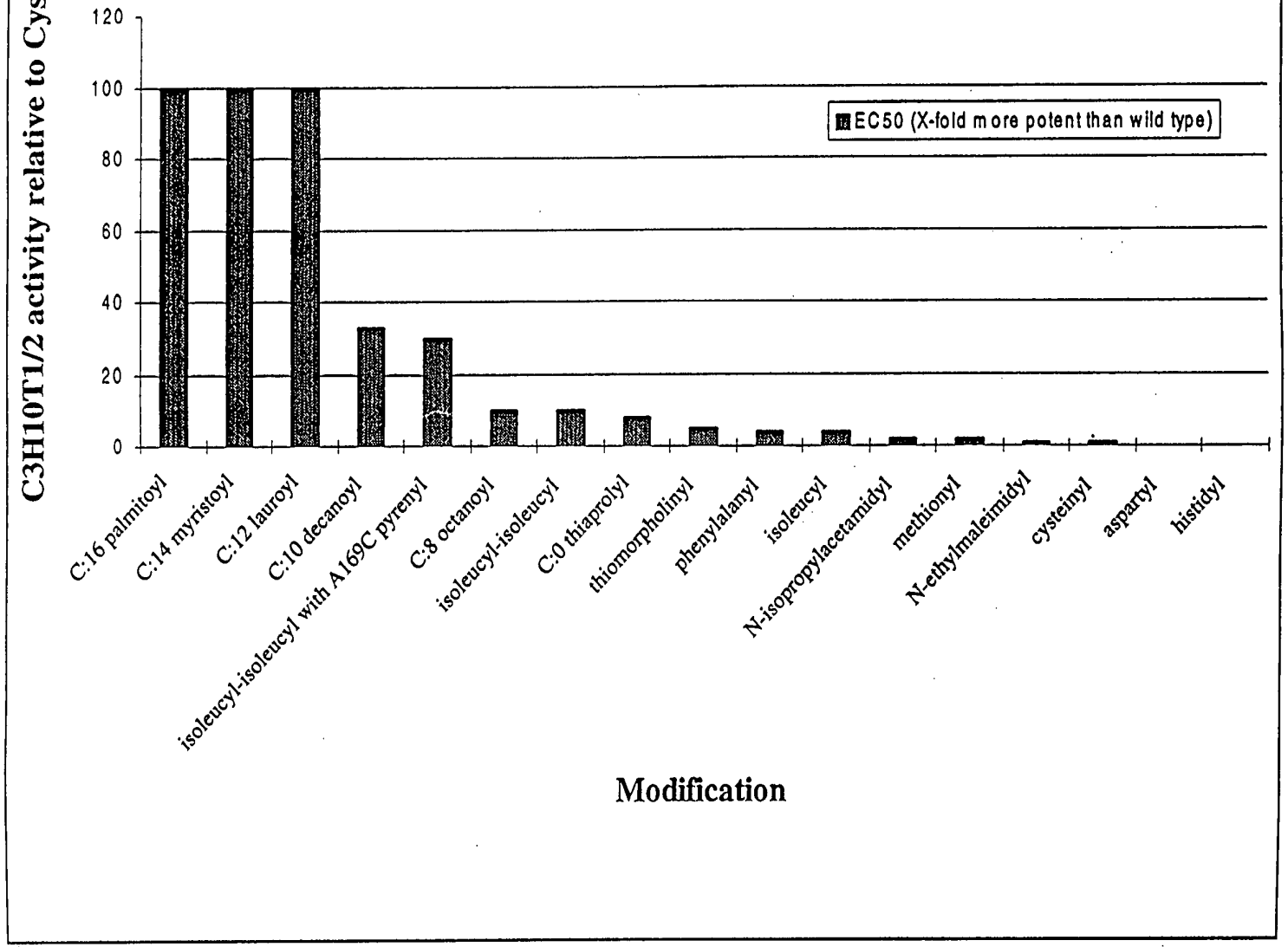


Figure 12

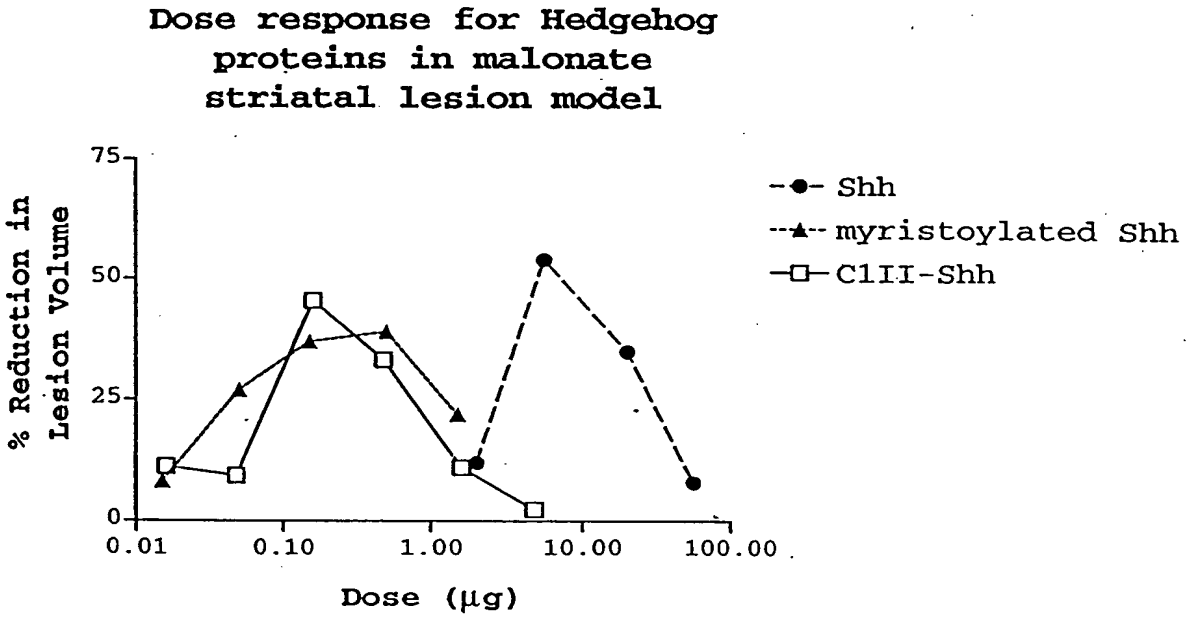
0056250 "60965/5650

Figure 13. Relative potency of various hydrophobically-modified forms of hedgehog in the C3H10T1/2 assay



0957660 .052603

Figure 14



09725.0056/P5650

Activities of maleimide modified sHh-N in10t1/2 cells

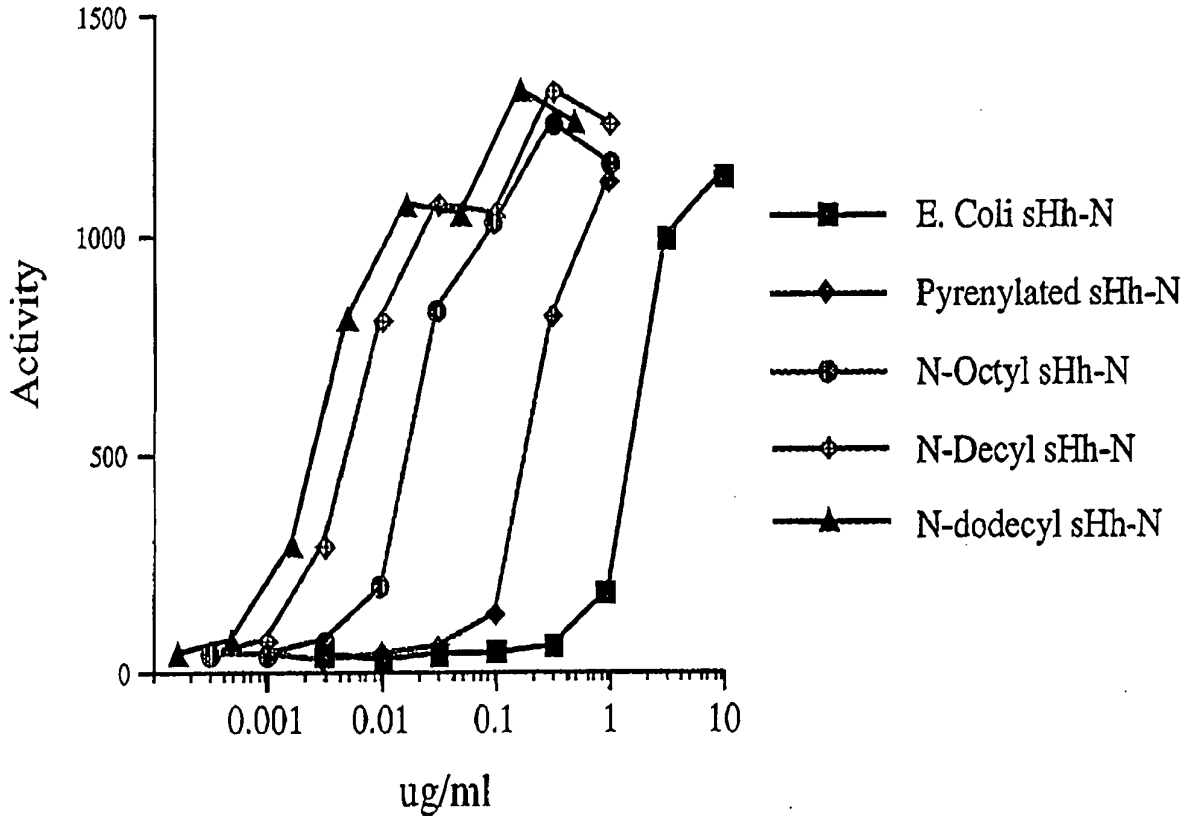


Figure 15

09579680 .052600